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## Document Number 2

Entry 2 of 2

File: USPT

May 25, 1999

DOCUMENT-IDENTIFIER: US 5907079 A

TITLE: MSH2 disrupted mice develop lymphomas

### BSPR:

The second locus, which appears to account for up to 20-30% of the HNPCC cases, was found to be located on chromosome 3p21 where mutations in the human MutL Homologue (hMLH1) gene were found to cosegregate with the disease (Bronner et al., Nature 368:258-261 (1994); Papadopoulos et al., Science 263:1625-1629 (1994)). These two genes code for homologues of the bacterial MutS and MutL proteins, which are essential components of the post-replication mismatch repair machinery (For a review see: Fishel and Kolodner, Curr. Op. Genet. Devel. 5:382-395 (1995)). Mutation of these genes in bacteria results in a generalized mutator (mut) phenotype that has been attributed to the absence of repair functions capable of recognizing mismatched nucleotides introduced into nascent DNA chains as a result of polymerase misincorporation errors. Such mismatches would subsequently lead to the passive accumulation of spontaneous mutations after a second round of DNA replication. Biochemical studies have shown that the MutS protein is involved in the initial mismatch recognition step and the MutL protein appears to link the excision repair machinery to mismatch recognition (Modrich, Ann. Rev. Genet 25:229-253 (1991)). These results support a direct role for mismatch repair functions in mutation avoidance.

### BSPR:

Two other MutL homologues have been described, HPMS1 and hPMS2, that are related to the S. cerevisiae PMS1 gene which was originally identified as a contributor to "Post-Meiotic Segregants": a genetic phenomenon that suggested unrepaired mismatched nucleotides following chromosomal recombination (Nicolaides et al., Nature 371:75-80 (1994)). Mutations of these genes have been found in sporadic colorectal tumors and, in the case of hPMS2, in HNPCC families and may account for an additional 5-10% of HNPCC cases (Parsons et al., Science 268:738-470 (1995)).

## BSPR:

The identification of hMSH2 and hMLH1 as genes in which mutations may predispose individuals to HNPCC, was facilitated by the observation that >85% of the tumors derived from these patients displayed instability of simple repetitive (microsatellite) sequences (Aaltonen et al., Science 260:812-816 (1993)). A similar nucleotide repeat instability was first observed in both *E. coli* and *S. cerevisiae* only when their respective mismatch repair genes were defective (Levinson and Gutman, Nucleic Acids Research 15:5313-5338 (1987); Strand et al., Nature 365:274-276 (1993)). Microsatellite instability has been observed in 5-85% of a variety of sporadic tumors (often termed: RER+ for replication error positive) suggesting that defects in mismatch repair or some related replication fidelity process may contribute widely to tumorigenesis (Ionov et al., Nature 363:558-561 (1993); Thibodeau et al., Science 260:816-819 (1993); Risinger et al., Cancer Res. 53:5100-5103 (1993); Young et al., Hum. Mutat. 2:351-354 (1993); Han et al., Cancer Res. 53:5087-5089 (1993); Peltomaki et al., Cancer Res. 53:5853-5855 (1993b); Gonzalez-Zulueta et al., Cancer Res. 53:5620-5623 (1993); Rhyu et al., Oncogene 9:29-32 (1994); Wada et al., Blood 83:3449-3456 (1994); Shridhar et al., Cancer Res. 54:2084-2087 (1994); Merlo et al., Cancer Res. 54:2098-2101 (1994); Wooster et al., Nature Genet. 6:152-156 (1994); Yee et al., Cancer Res. 54:1641-1644 (1994); Burks et al., Oncogene 9:1163-1166 (1994); Schoenberg et al., Biochem. Biophys. Res. Commun. 198:74-80 (1994); Honchel et al., Cancer Res. 54:1159-1563 (1994); Shibata et al., Nature Genet. 6:273-281 (1994); and Aaltonen et al., Cancer Res. 54:1645-1648 (1994)). In addition to microsatellite instability, cell lines that contain mutations in hMSH2 or hMLH1 are also defective for mismatch repair in vitro (Umar et al., J. Biol. Chem. 259:1-4 (1994a); Parsons et al., Science 75:1227-1236 (1993)), they display a generalized increase in spontaneous mutation frequency (Bhattacharyya et al., Proc. Natl. Acad. Sci. USA 91:6319-6323 (1994)) and are resistant to alkylating agents (Kat et al., Proc. Natl. Acad. Sci. USA 90:6424-6428 (1993)). The connection of microsatellite instability to a generalized mutator phenotype has suggested that the detection of such changes might be used as a convenient molecular diagnosis of a mismatch repair defect and a mutator phenotype in clinically presented tumors.

## BSPR:

In both bacteria and yeast, MutS and its homologues play additional roles in genetic recombination. Mutational studies have shown that recombination between closely spaced markers is increased (Fishel et al., J. Mol. Biol. 188(2):147-157 (1986); Feinstein and Low, Genetics 113:13-33 (1986); Jones et al., Cell 50:621-626 (1987)) and the length of DNA tracts exchanged between recombining chromosomes is reduced (Alani et al., Genetics 137:19-39

(1994)) in MutS (or MSH2) deficient cells. In addition, the tolerance of heterologous DNA sequence in recombining chromosomes is substantially increased in such bacterial or yeast cells (Rayssiguier et al., Nature 342(6248):396-401 (1989); Selva et al., Genetics 139:1175-1188 (1995)). These results suggest that mismatched nucleotides formed during genetic recombination provide a target for mismatch repair functions which, in the case of multiple mismatches, results in abortion of the recombination process (Radman, Genome 31(1):68-73 (1989); and Worth et al., Proc. Nat. Acad. Sci. USA 91:3238-3241 (1994)). This later observation has particular relevance to carcinogenesis since large scale rearrangements between non-homologous and/or partially homologous chromosomal sequences are a hallmark of tumor cells and may be indicative of widespread reduced-fidelity recombination processes.

#### BSPR:

Although the precise function of hMLH1 is poorly understood, hMSH2 has been purified and found to bind insertion/deletion loop-type (IDL) mismatched nucleotides with high affinity, and the single base pair G/T mismatch with lower affinity (Fishel et al., Science 266:1403-1405 (1994a); Fishel et al., Cancer Res. 54:5539-5542 (1994b)). IDL mismatched nucleotides have been proposed as an intermediate in microsatellite instability (Kunkel, Nature 365:207-208 (1993)). Furthermore, biochemical reconstitution studies that examine mismatch repair functions in vitro (Umar et al., J. Biol. Chem. 259:1-4 (1994a); Umar et al., Science 266:814-816 (1994b)), have resulted in the purification of two activities that appear to complement extracts derived from cell lines with known mutations in hMSH2 and hMLH1. Interestingly, both these purified complementing fractions consist of tightly complexed heterodimers. Protein extracts of the LoVo cell line, which contains deletions of both hMSH2 alleles, are complemented by a heterodimer that consists of hMSH2 (105 kDa) and a 160 kDa protein (P. Modrich, Ann. Rev. Genet. 25:229-253 (1991)), that has been cloned and identified as another MutS homologue a GT binding protein (GTBP/P160) and which has been found to co-purify as a heterodimer with hMSH2). There is some suggestion that the hMSH2/GTBP/160 heterodimer may bind mismatched nucleotides with a much higher affinity than either of the corresponding individual proteins. Protein extracts of the HCT116 cell line, which contains a deletion and a non-sense mutation of the hMLH1 alleles, are complemented by a heterodimer of hMLH1 (84 kDa) and hPMS2 (110 kDa) (Li and Modrich, Proc. Nat. Acad. Sci. USA 92:1950-1954 (1995)).

#### BSPR:

There are multiple possibilities by which faulty mismatch repair genes could result in the development of cancer (Fishel and Kolodner, Curr. Op. Genet. Devel. 5:382-395 (1995)). It has been hypothesized that cells with repair

defects might have elevated rates of mutations (Loeb, Cancer Res. 51:3075-3079 (1991)). The accumulation of mutations could result in growth control defects--such as by interfering with check-point control mechanisms, tumor suppressors, or oncogenes that cause mutant cells to progress to malignancy. It is clear that an appropriate animal model is needed to investigate the possible role(s) of mismatch repair in tumorigenesis and to provide systems for testing of therapeutic interventions for the treatment of cancer and other diseases associated with mismatch repair deficiencies.

BSPR:

Although mutations in the human MSH2 gene co-segregate with malignant disease in a number of HNPCC kindreds, it has remained debatable whether mismatch repair is involved directly in the onset of tumorigenesis. Described below are mice having one or more disrupted MSH2 alleles. These mice are useful for the study of the role of mismatch repair in oncogenesis and as screening tools for suspected carcinogens and chemotherapeutic agents. While these mice are fertile and viable through at least 3 generations, they succumb to tumors at an early age with high frequency, supporting a role for MSH2 in tumorigenesis.

BSPR:

The present invention is directed to a non-human mammal, preferably a rodent having a disrupted MSH2 gene. The disruption may be introduced into the endogenous MSH2 gene by homologous recombination resulting in the insertion of a marker sequence into the gene, and preferably an exon of the gene. The marker sequence comprises, for example a gene encoding resistance to an antibiotic such as neomycin, although other selectable markers are also comprehended by the invention. Preferably, the selectable marker is under the transcriptional control of a promoter capable of functioning in embryonic stem cells. One such promoter is the mouse phosphoglycerate kinase (PGK) promoter although other such promoters known to those of ordinary skill in the art may be used (e.g., the herpes simplex thymidine kinase gene promoter).

BSPR:

The invention is further directed to a mouse having a disrupted MSH2 gene wherein the disruption is introduced using a DNA construct comprising a mouse DNA corresponding to at least part of exon 11 of a human MSH2 gene and a neomycin resistance gene cassette comprising the mouse PGK promoter operatively linked to a neomycin resistance gene (PGK-neo), and wherein PGK-neo is inserted into the MSH2 gene in an antisense orientation via homologous recombination.

BSPR:

The mammals of the present invention are also useful in the screening of antineoplastic agents, and more particularly they may be useful for screening

chemotherapeutic agents for the treatment of lymphomas or other tumors resulting from defective mismatch repair in the subject animals. The method comprises administering the candidate antineoplastic agent to mouse having a disrupted MSH2 gene and a tumor, particularly a lymphoma, followed by monitoring the mouse for tumor associated morbidity and mortality, and wherein a successful chemotherapeutic agent decreases the morbidity and/or extends the life expectancy of the mouse. Cells derived from the mice of the present invention may also be used to screen for potential therapeutic agents which preferentially inhibit growth of mismatch repair deficient cells which may give rise to tumors.

DEPR:

The mammals of the present invention comprise a disruption of an MSH2 gene of the mammal or the disruption of a homolog of the MSH2 gene (encoding mismatch repair proteins). The general strategy utilized to produce the mammals of the present invention involves the preparation of a targeting construct comprising DNA sequences homologous to the endogenous gene to be disrupted. The targeting construct is then introduced into embryonic stem cells (ES cells) whereby it integrates into and disrupts the endogenous MSH2 gene or homolog thereof. After selection of ES cells for the disruption, the selected cells are implanted into an embryo at the blastocyst stage.

DEPR:

The targeting construct is introduced into a cell containing the endogenous target gene. The targeting construct can then recombine with one allele of the endogenous target gene (the MSH2 gene in the present case) and such recombination of the targeting construct can prevent or interrupt expression of the full-length endogenous MSH2 protein. Integration of the targeting construct into embryonic stem cell DNA is detected by selecting the cells for expression of the marker sequence. Introduction of the MSH2 targeting construct into the chromosomal DNA encoding MSH2 may occur via homologous recombination (i.e., via regions of the MSH2 targeting construct that are homologous or complementary to endogenous MSH2 DNA).

DEPR:

Offspring (chimaeras) that are born to the foster mother may be screened initially for mosaic coat color or another phenotype marker where the phenotype selection strategy has been employed. In addition, or as an alternative, chromosomal DNA obtained from tail tissue of the offspring may be screened for the presence of the targeting construct using Southern blots and/or PCR which will not only detect the presence of the targeting construct but also the chromosomal location of the construct. The offspring that are positive for homologous recombination of the MSH2 targeting construct will typically be a mosaic

of wild-type cells derived from the blastocyst and heterozygous cells, derived from injected ES cells.

DEPR:

If animals homozygous for the disruption are desired, they can be prepared by crossing heterozygous agoutis carrying the disruption in their germ line to each other; such crosses may generate animals homozygous for the disruption. Germ line transmission can be tested by crossing the chimera with a parental or other strain and the offspring screened for the presence of the targeting construct. Mammals homozygous for the disruption may be identified by Southern blotting of equivalent amounts of genomic DNA from mammals that are the product of this cross, as well as mammals of the same species that are known heterozygotes, and wild-type mammals. Probes to screen the Southern blots for the presence of the targeting construct in the genomic DNA can be designed as described below.

DEPR:

The targeting construct described in Example 1 was linearized by digestion with HindIII prior to introduction into E14 cells by electroporation. E14 embryonic stem cells were transfected with 20 .mu.g of linearized targeting construct DNA per 3.times.10.sup.6 embryonic stem cells by electroporation using a Bio-Rad Gene Pulser, 0.34 kV, 0.25 mF (Hercules, Calif. and Melville, N.Y.). Approximately, 28 hours after transfection G418 selection (280 .mu.g/ml) was started, and G418 resistant colonies were obtained after 10 days of selection. A polymerase chain reaction method was used to screen G418 resistant E14 cells for homologous recombination events according to a method described by Fung-Leung et al., Cell 65:443-449 (1991).

DEPR:

PCR screening of ES cell DNA for homologous recombination events utilized a primer specific for the neomycin resistance gene cassette 5'-GCCAGCTCATTCCTCCACTC-3' (SEQ ID NO: 5) and an outside primer specific for the MSH2 gene 5'-CACCACCACAGCTCTCTTGT-3' (SEQ ID NO: 6) located upstream of the construct. (See FIG. 2, pair of arrows.) Following an initial denaturation step (94.degree. C. for 10 min), 40 cycles of PCR were performed using the following conditions: 94.degree. C. for 1 min, 62.degree. C. for 30 sec, 72.degree. C. for 2 min. The purity of positive clones was subsequently examined by Southern blot analysis of EcoRI digested genomic DNA after hybridization with a probe flanking the targeting vector (See, FIG. 2) as well as a neomycin specific probe.

DEPR:

Microsatellite instability has been used as a hallmark of mismatch repair defects to investigate the possibility of mismatch repair defects. Various tissues were harvested from MSH2.sup.+/+, MSH2.sup.+/-, MSH2.sup.-/- mice for

microsatellite analysis. DNA was extracted from fresh frozen tissues using Trizol (Gibco, BRL-Life Technologies, Bethesda, Md.) according to the manufacturer's instructions. For paraffin embedded tissue biopsy specimens, 5  $\mu$ m-thick sections were examined by hematoxylin and eosin staining and areas corresponding to normal and tumor histology were marked on the matched biopsy specimens. DNA was extracted as crude preparations from these specimens using a proteinase K lysis mix (10 mM Tris pH 8.0, 100 mM KCl, 2.5 mM MgCl<sub>2</sub>, 0.45% Tween-20 and proteinase K). The sections were briefly homogenized in the lysis mix and digested for 1 hr at 65.degree. C. followed by 10 min. at 95.degree. C. DNA was stored at -20.degree. C.

DEPR:

By virtue of their defects in mismatch repair, the animals of the present invention may accumulate DNA damage at a rate faster than wildtype mice thereby mimicking age-related accumulation of DNA damage. Using such animals makes it possible to detect carcinogens that would otherwise have been missed using older animals.

DEPR:

The same types of studies may also be performed on mice of different genetic backgrounds (e.g. RAG-1) into which the disrupted MSH2 alleles have been introduced. This would provide the opportunity to determine the effect of mismatch repair defects on the development of tumors in mice with different susceptibility to tumors.

CLPR:

2. The mouse of claim 1, wherein said disruption is introduced into said MSH2 gene by homologous recombination of said marker into an exon of said MSH2 gene.

ORPL:

Alani et al., "Interaction Between Mismatch Repair and Genetic Recombination in *Saccharomyces cerevisiae*," Genetics, 137:19-39 (May, 1994).

ORPL:

Baker et al., "Male Mice Defective in the DNA Mismatch Repair Gene PMS2 Exhibit Abnormal Chromosome Synapsis in Meiosis," Cell, 82:309-319 (Jul. 28, 1995).

ORPL:

Bronner et al., "Mutation in the DNA Mismatch Repair Gene Homologue hMLH 1 is Associated with Hereditary Non-Polyposis Colon Cancer," Nature, 368:258-261 (Mar. 17, 1994).

ORPL:

de Wind et al., "Inactivation of the Mouse Msh2 Gene Results in Mismatch Repair Deficiency, Methylation Tolerance, Hyperrecombination, and Predisposition to Cancer," Cell, 82:321-330 (Jul. 28, 1995).



ORPL:

Fishel and Kolodner, "Identification of Mismatch Repair Genes and Their Role in the Development of Cancer," Curr. Op. Genet. Devel., 5:382-395 (1995).

ORPL:

Jones et al., "Mismatch Repair and Recombination in E. coli," Cell, 50:621-626 (Aug. 14, 1987).

ORPL:

Kat et al., "An Alkylation-Tolerant, Mutator Human Cell Line is Deficient in Strand-Specific Mismatch Repair," Proc. Nat'l Acad. Sci., USA, 90:6424-6428 (Jul., 1993).

ORPL:

Li and Modrich, "Restoration of Mismatch Repair to Nuclear Extracts of H6 Colorectal Tumor Cells By a Heterodimer of Human MutL Homologs," Proc. Nat'l Acad. Sci., USA, 92:1950-1954 (Mar., 1995).

ORPL:

Modrich, P., "Mechanisms and Biological Effects of Mismatch Repair," Ann. Rev. Genet., 25:229-253 (1991).

ORPL:

Nystrom-Lahti et al., "Mismatch Repair Genes on Chromosome 2p and 3p Account for a Major Share of Hereditary Nonpolyposis Colorectal Cancer Families Evaluable by Linkage," Am. J. Hum. Genet., 55:659-665 (1994).

ORPL:

Parsons et al., "Hypermutability and Mismatch Repair Deficiency in RER.sup.+ Tumor Cells," Cell, 75:1227-1236 (1993).

ORPL:

Parsons et al., "Mismatch Repair Deficiency in Phenotypically Normal Human Cells," Science, 268:738-770 (May 5, 1995).

ORPL:

Radman, M., "Mismatch Repair and the Fidelity of Genetic Recombination," Genome, 31:68-73 (1989).

ORPL:

Rayssiguier et al., "The Barrier to Recombination Between Escherichia coli and Salmonella typhimurium is Disrupted in Mismatch-Repair Mutants," Nature, 343(6248):396-401 (Nov. 23, 1989).

ORPL:

Selva et al., "Mismatch Correction Acts as a Barrier to Homeologous Recombination in Saccharomyces cerevisiae," Genetics, 139:1175-1188 (Mar., 1995).

ORPL:

Strand et al., "Destabilization of Tracts of Simple

Repetitive DNA in Yeast by Mutations Affecting DNA  
Mismatch Repair, " Nature, 365:274-276 (Sep. 16, 1993).

## ORPL:

Thompson et al., "Germ Line Transmission and Expression of  
a Corrected HPRT Gene Produced by Gene Targeting in  
Embryonic Stem Cells," Cell, 56:313-321 (Jan. 27, 1989).

## ORPL:

Umar et al., "Defective Mismatch Repair in Extracts of  
Colorectal and Endometrial Cancer Cell Lines Exhibiting  
Microsatellite Instability," J. Biol. Chem.,  
269(20):14367-14370 (1994).

## ORPL:

Worth et al., "Mismatch Repair Proteins MutS and MutL  
Inhibit RecA-Catalyzed Strand Transfer Between Diverged  
DNAs," Proc. Nat'l. Acad. Sci., USA, 91:3238-3241 (Apr.,  
1994).

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## Document Number 9

Entry 9 of 43

File: USPT

Jul 13, 1999

DOCUMENT-IDENTIFIER: US 5922855 A

TITLE: Mammalian DNA mismatch repair genes MLH1 and PMS1

## ABPL:

We have discovered two human genes, hMLH1 and hPMS1, each of which apparently encodes for a protein involved in DNA mismatch repair. The hMLH1 gene encodes for a protein which is homologous to the bacterial DNA mismatch repair protein MutL, and is located on human chromosome 3p21.3-23. We believe that mutations in the hMLH1 gene cause hereditary non-polyposis colon cancer (HNPCC) in some individuals based upon the similarity of the hMLH1 gene product to the yeast DNA mismatch repair protein MLH1, the coincident location of the hMLH1 gene and the HNPCC locus on chromosome 3, and hMLH1 missense mutations in affected individuals from a chromosome 3-linked HNPCC family. The human hPMS1 gene is homologous to the yeast DNA mismatch repair gene PMS1, and is located on human chromosome 7q. We believe that the hPMS1 gene is a strong candidate for HNPCC testing because the yeast proteins MLH1 and PMS1 have been shown to be involved in the same DNA repair pathway and because hMLH1 and hMSH2 have both been implicated in HNPCC families. The most immediate use for hMLH1 and hPMS1 will be in screening tests on individuals who are members of families which exhibit high frequencies of early onset cancer. We have also isolated and sequenced mouse MLH1 and PMS1 genes. We have produced chimeric mice with a mutant form of the PMS1 gene that will enable us to derive mice that are heterozygous or homozygous for mutation in mPMS1. These mice will be useful for cancer research. We have also produced and isolated antibodies directed to hPMS1 which are useful in assays to detect the presence of protein in tumor samples.

## PCPR:

This application is a continuation-in-part from U.S. patent application Ser. No. 08/168,877, titled: STRUCTURES FOR AND METHODS OF USING HUMAN DNA MISMATCH REPAIR GENES MLH1 AND MLH2 TO SCREEN FOR CANCER RISK, filed on Dec. 17, 1993, abandoned, the entirety of which is hereby incorporated by reference.

## BSPR:

Based on our knowledge of DNA mismatch repair mechanisms in bacteria and yeast including conservation of mismatch repair genes, we reasoned that, in addition to hMSH2, other human DNA mismatch repair homologs should exist. Further, mutations in such homologs affecting protein function, would be likely to cause genetic instability, possibly leading to an increased risk of certain forms of human cancer. Therefore, an important objective of our work has been to identify other candidate human genes which are useful for screening and identifying individuals who are at elevated risk of developing cancer.

## BSPR:

We have isolated and sequenced two human genes, hPMS1 and hMLH1 each of which we believe encodes for a protein involved in DNA mismatch repair. Our studies strongly support an association between a mutation in hMLH1 and susceptibility to HNPCC. We believe mutations in hPMS1 may also result in an elevated risk of cancer. We believe our characterization and localization of hMLH1 and hPMS1 will be useful in the diagnosis, prevention and treatment of cancer. The most immediate use will be in screening tests on individuals who are members of families which exhibit an unusually high frequency of early onset cancer, for example, HNPCC.

## BSPR:

Here we describe the isolation, sequence characterization and chromosomal map positions of hMLH1 and hPMS1. Each of these genes, based upon its similarity to bacterial and yeast genes of known function, is likely to have a role in repairing DNA replication errors that invariably occur during each cell division. The DNA correction process appears to be highly conserved from bacteria to humans, and is commonly referred to as DNA mismatch repair. Based upon studies in bacteria and yeast and the recent finding that mutations in a mismatch repair gene underlie certain forms of human cancer, inheritance of a mutation affecting protein function in either hMLH1 or hPMS1 is expected to raise mutation rates, and therefore predispose such individuals to multiple forms of cancer.

## DEPR:

We have discovered two mammalian genes which are apparently involved in DNA mismatch repair. One of the genes, PMS1, encodes a protein which is homologous to the yeast DNA mismatch repair protein PMS1. We have mapped the locations of PMS1 to human chromosome 7q and to mouse chromosome 5, band G. The other gene, MLH1 (MutL Homolog) encodes a protein which is homologous to the bacterial DNA mismatch repair protein MutL. We have mapped the locations of MLH1 to human chromosome 3p21.3-23 and to mouse chromosome 9, band E.

## DEPR:

Recent studies <sup>1,2</sup> have demonstrated involvement of a human DNA mismatch repair gene homolog, hMSH2, on chromosome 2p in HNPCC. Based upon linkage data, a second HNPCC locus has been assigned to chromosome 3p21-23. <sup>3</sup> Examination of tumor DNA from the chromosome 3-linked kindreds revealed dinucleotide repeat instability similar to that observed for other INPCC families. <sup>6</sup> and several types of sporadic tumors. <sup>7-10</sup> Because dinucleotide repeat instability is characteristic of a defect in DNA mismatch repair, <sup>5, 11, 12</sup> we reasoned that HNPCC linked to chromosome 3p21-23 could result from a mutation in a second DNA mismatch repair gene.

DEPR:

Based on several criteria, we suggest that the observed C to T substitution in the coding region of hMLH1 represents the mutation that is the basis for HNPCC in Family 2. <sup>3</sup> First, DNA sequence and ASO analysis did not detect the C to T substitution in 74 unrelated individuals. Thus, the C to T substitution is not simply a polymorphism. Second, the observed C to T substitution is expected to produce a serine to phenylalanine change at position 44 (See FIG. 9). This amino acid substitution is a non-conservative change in a conserved region of the protein (FIGS. 3 and 9). Secondary structure predictions using Chou-Fasman parameters suggest a helix-turn-beta sheet structure with position 44 located in the turn. The observed Ser to Phe substitution, at position 44 lowers the prediction for this turn considerably, suggesting that the predicted amino acid substitution alters the conformation of the hMLH1 protein. Therefore, we propose that hMLH1 represents a second DNA mismatch repair gene that is involved in HNPCC. At present, we have no direct evidence that the hMLH1 gene is involved in the correction of DNA mispairs. In bacteria and yeast, a mutation affecting DNA mismatch repair causes comparable increases in the rate of spontaneous mutation including additions and deletions within dinucleotide repeats. <sup>4,5,11,13,14,15,16</sup> In humans, mutation of hMSH2 is the basis of chromosome-2 HNPCC, <sup>1,2</sup> tumors of which show microsatellite instability and an apparent defect in mismatch repair. <sup>12</sup> Chromosome 3-linked HNPCC is also associated with instability of dinucleotide repeats. <sup>3</sup> Combined with these observations, the high degree of conservation between the human MLH1 protein and the yeast DNA mismatch repair protein MLH1 suggests that hMLH1 is likely to function in DNA mismatch repair. During isolation of the hMLH1 gene, we identified the hPMS1 gene. This observation suggests that mammalian DNA mismatch repair, like that in yeast, <sup>4</sup> may require at least two MutL-like proteins.

DEPR:

Our recent map position and mutation data strongly suggest that the hMLH1 gene is the HNPCC locus that, based upon linkage studies, maps to chromosome 3p. <sup>3</sup> The hPMS1 on 7q is, a priori, also a strong candidate to represent

HNPCC that maps to neither 2p or 3p.1-3,6 At present, our collaborators in Sweden are checking such "unlinked" HNPCC families for linkage to 7q. Clearly, our data indicate that the hMLH1 gene has significant utility in the screening and diagnosis of hereditary human cancer. Further studies are required to fully determine the utility of the hPMS1 gene in human cancer risk screening. We would like to point out, however, that several observations make the hPMS1 gene a strong DNA mismatch repair gene candidate and hence a possible player in human cancer. These observations include: 1) the involvement of mismatch repair gene homologs, hMSH2 and hMLH1, in HNPCC;.sup.1,2 2) the close similarity of the human and yeast PMS1 proteins 3) the role of the yeast PMS1 protein in DNA mismatch repair;.sup.4 and 4) our published genetic and unpublished biochemical data strongly suggesting that the yeast PMS1 and MLH1 proteins act as a heteromeric complex during DNA mismatch repair.

**DEPR:**

Using the procedures discussed above with reference to FIG. 1, we isolated and sequenced the mouse PMS1 gene, as shown in FIG. 15. This cDNA sequence encodes a predicted protein of 864 amino acids, as shown in FIG. 16, where it is compared to the predicted amino acid sequence for hPMS1. The degree of identity between the predicted mouse and human PMS1 proteins is high, as would be expected between two mammals. Similarly, as noted above, there is strong similarity between the human PMS1 protein and the yeast DNA mismatch repair protein PMS1, as shown in FIG. 11. The fact that yeast PMS1 and MLH1 function in yeast to repair DNA mismatches, strongly suggests that human and mice PMS1 and MLH1 are also mismatch repair proteins.

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## Document Number 10

Entry 10 of 14

File: USPT

Mar 31, 1998

DOCUMENT-IDENTIFIER: US 5733761 A

TITLE: Protein production and protein delivery

## DEPR:

Several lines of evidence suggest that 3'-overhanging ends are involved in certain homologous recombination pathways of *E. coli*, bacteriophage, *S. cerevisiae* and *Xenopus laevis*. In *Xenopus laevis* oocytes, molecules with 3'-overhanging ends of several hundred base pairs in length underwent recombination with similarly treated molecules much more rapidly after microinjection than molecules with very short overhangs (4 bp) generated by restriction enzyme digestion. In yeast, the generation of 3'-overhanging ends several hundred base pairs in length appears to be a rate limiting step in meiotic recombination. No evidence for an involvement of 3'-overhanging ends in recombination in human cells has been reported, and in no case have modified DNA substrates of any sort been shown to promote targeting (one form of homologous recombination) in any species. In human cells, the effect of 3'-overhanging ends on targeting is untested. The experiment described in the following example and Example 1c suggests that 5'-overhanging ends are effective for stimulating targeting in primary, secondary and immortalized human fibroblasts.

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## Document Number 11

Entry 11 of 21

File: USPT

Sep 8, 1998

DOCUMENT-IDENTIFIER: US 5804191 A

TITLE: Sperm as immunogen carriers

## BSPR:

Gene therapy involves administering DNA to cells to permanently alter their protein expression phenotype and that of their progeny. Recent advances in gene therapy indicate that it may become a viable treatment option for cancer and a number of genetic diseases, and that vaccination with DNA might prove useful for immunization. Studies with DNA carried by sperm now suggest that gene therapy with ova and embryos may also allow for correction of genetic defects in germ line cells via fertilization. As with the vaccines and contraceptives discussed above, many problems associated with gene therapy relate to the delivery vectors.

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<a href="#">Full</a>	<a href="#">Title</a>	<a href="#">Citation</a>	<a href="#">Front</a>	<a href="#">Review</a>	<a href="#">Classification</a>
<a href="#">Date</a>	<a href="#">Reference</a>	<a href="#">Claims</a>	<a href="#">KWIC</a>		

## Document Number 14

Entry 14 of 21

File: USPT

Aug 4, 1998

DOCUMENT-IDENTIFIER: US 5789215 A

TITLE: Gene targeting in animal cells using isogenic DNA constructs

## DEPR:

The present invention can be used with essentially any cell into which DNA can be introduced. As discussed in the following section, there are a variety of methods applicable for introducing DNA into animal cells. The choice of cell type will depend on the particular goal of the site-directed mutagenesis. For example, embryonic stem cells or zygotes may be targeted for generating modified animals; whereas both germ-line and somatic cells may be usefully targeted for gene therapy. The choice of cells may also affect (or be affected by) the choice of transformation technique, as discussed below. Growth and manipulation of the cells can be performed using standard procedures as described in Hogan, B., et al, Manipulating the Mouse Embryo, Cold Spring Harbor, N.Y. (1986).

<a href="#">Main Menu</a>	<a href="#">Search Form</a>	<a href="#">Result Set</a>	<a href="#">ShowS Numbers</a>	<a href="#">Edit S Numbers</a>	<a href="#">Referring Patents</a>
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<a href="#">Full</a>	<a href="#">Title</a>	<a href="#">Citation</a>	<a href="#">Front</a>	<a href="#">Review</a>	<a href="#">Classification</a>
<a href="#">Date</a>	<a href="#">Reference</a>	<a href="#">Claims</a>	<a href="#">KWIC</a>		

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## Document Number 3

Entry 3 of 43

File: USPT

Jan 4, 2000

DOCUMENT-IDENTIFIER: US 6010907 A

TITLE: Eukaryotic use of non-chimeric mutational vectors

## BSPR:

Many of the genes required for mismatch repair in yeast and humans have been cloned based on homology with the E. coli mismatch repair genes. Kolodner, R., 1996, Genes & Development 10, 1433-1442. Cells having defective mismatch repair genes show genetic instability, termed Replication Error (RER), particularly evident in microsatellite DNA, and malignant transformation. Extracts of RER cells do not have mismatch repair activity. Umar, A., et al., J. Biol. Chem. 269, 14367-14370.

## DRPR:

Chimeraplasty is an increasingly important process for the treatment of human disease and the development of useful, genetically engineered plant and animal strains. The development of improved recombinagenic oligonucleotides has been greatly facilitated by the use of bacterial testing systems, which give rapid and quantitative results as described in commonly assigned regular U.S. application Ser. No. 09/078,063, filed May 12, 1998, entitled "Non-Chimeric Mutational Vectors" by R. Kumar et al., and U.S. Provisional Application No. 60/085,191 filed May 12, 1998, entitled "Heteroduplex Mutational Vectors and Use Thereof in Bacteria" by Kumar et al., (hereafter collectively "Kumar") filed on even date herewith, which are hereby incorporated by reference in its entirety. The techniques of Kumar do not address whether the optimal recombinagenic oligonucleotides in bacterial systems are also optimal in eukaryotes. The prior art techniques of in vivo and cell-culture chimeraplasty are not designed for rapid quantitative analysis and are unable to utilize the same recombinagenic oligonucleobases and DNA targets as used in the bacterial systems. Accordingly, an objective of the present invention is an assay that can use DNA targets and recombinagenic oligonucleobases designed for bacterial systems to rapidly evaluate the compatibility between different types of recombinagenic oligonucleotides and the recombination and repair enzymes of different phyla, e.g., do the recombination and mismatch repair

enzymes of bacteria, plants, insects and mammals have differing substrate preferences?

DEPR:

A cell-free enzyme mixture that lacks mismatch repair can be obtained from extracts of mutant cells having the replication error phenotype. Umar et al., 1994, J. Biol. Chem. 269, 14367. The cell line LoVo has deleted both alleles of the human MutS homolog (MSH2) and is suitable as a source of strand transfer activity without mismatch repair activity.

ORPL:

Glazer, P. M., et al, "DNA mismatch repair detected in human cell extracts", Jan., 1987, Molecular and Cellular biology, 7(1), 218-224.

Main Menu	Search Form	Result Set	Show S Numbers	Edit S Numbers	Referring Patents				
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L9 ANSWER 5 OF 10 MEDLINE DUPLICATE 2  
TI The \*\*\*mismatch\*\*\* \*\*\*repair\*\*\* system contributes to meiotic sterility in an interspecific yeast \*\*\*hybrid\*\*\* .  
AN 96203091 MEDLINE  
DN 96203091  
TI The \*\*\*mismatch\*\*\* \*\*\*repair\*\*\* system contributes to meiotic sterility in an interspecific yeast \*\*\*hybrid\*\*\* .  
AU Hunter N; Chambers S R; Louis E J; Borts R H  
CS Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, UK.  
SO EMBO JOURNAL, (1996 Apr 1) 15 (7) 1726-33.  
Journal code: EMB. ISSN: 0261-4189.  
CY ENGLAND: United Kingdom  
DT Journal; Article; (JOURNAL ARTICLE)  
LA English  
FS Priority Journals  
EM 199608

AB The \*\*\*mismatch\*\*\* \*\*\*repair\*\*\* system is the major barrier to genetic recombination during interspecific sexual conjugation in prokaryotes. The existence of this anti-recombination activity has implications for theories of evolution and the isolation of species. To determine if this phenomenon occurs in eukaryotes, the effect of a deficiency of \*\*\*mismatch\*\*\* \*\*\*repair\*\*\* on the meiotic sterility of an interspecific \*\*\*hybrid\*\*\* of *Saccharomyces cerevisiae* and the closely related species *Saccharomyces paradoxus* was examined. The results demonstrate that the rare viable spores from these \*\*\*hybrids\*\*\* have high frequencies of aneuploidy and low frequencies of genetic exchange. \*\*\*Hybrids\*\*\* lacking \*\*\*mismatch\*\*\* \*\*\*repair\*\*\* genes PMS1 or MSH2 display increased \*\*\*meiotic\*\*\* \*\*\*recombination\*\*\*, decreased chromosome non-disjunction and improved spore viability. These observations are consistent with the proposal that the \*\*\*mismatch\*\*\* \*\*\*repair\*\*\* system is an element of the genetic barrier between eukaryotic species. We suggest that an anti-recombination activity during meiosis contributes towards the establishment of post-zygotic species barriers.

L45 ANSWER 3 OF 10 MEDLINE DUPLICATE 1  
TI \*\*\*Mismatch\*\*\* \*\*\*repair\*\*\* in *Schizosaccharomyces pombe* requires the mutL homologous gene pms1: molecular cloning and functional analysis.  
AN 97403304 MEDLINE  
DN 97403304  
TI \*\*\*Mismatch\*\*\* \*\*\*repair\*\*\* in *Schizosaccharomyces pombe* requires the mutL homologous gene pms1: molecular cloning and functional analysis.  
AU Schar P; Baur M; Schneider C; Kohli J  
CS Institute of Medical Radiobiology, University of Zurich, Switzerland.  
schaer@imr.unizh.ch  
SO GENETICS, (1997 Aug) 146 (4) 1275-86.  
Journal code: FNH. ISSN: 0016-6731.  
CY United States  
DT Journal; Article; (JOURNAL ARTICLE)  
LA English  
FS Priority Journals  
OS GENBANK-X96581; GENBANK-U13696; GENBANK-U28724; GENBANK-M29688; GENBANK-U13695; GENBANK-U07343; GENBANK-U07187

EM 199801

EW 19980104

AB Homologues of the bacterial mutS and mutL genes involved in DNA \*\*\*mismatch\*\*\* \*\*\*repair\*\*\* have been found in organisms from bacteria to humans. Here, we describe the structure and function of a newly identified Schizosaccharomyces pombe that encodes a predicted amino acid sequence of 794 residues with a high degree of homology to MutL related proteins. On the basis of its closer relationship to the eukaryotic "PMS" genes than to the "MLH" genes, we have designated the S. pombe homologue pms1. Disruption of the pms1 gene causes a significant increase of spontaneous mutagenesis as documented by reversion rate measurements. Tetrad analyses of crosses homozygous for the pms1 mutation reveal a reduction of spore viability from > 92% to 80% associated with a low proportion (approximately 50%) of meioses producing four viable spores and a significant, allele-dependent increase of the level of post-meiotic segregation of genetic marker allele pairs. The mutant phenotypes are consistent with a general function of pms1 in correction of mismatched base pairs arising as a consequence of DNA polymerase errors during DNA synthesis, or of \*\*\*hybrid\*\*\* DNA formation between homologous but not perfectly complementary DNA strands during \*\*\*meiotic\*\*\* \*\*\*recombination\*\*\*.

L9 ANSWER 3 OF 10 MEDLINE

DUPLICATE 1

TI \*\*\*Mismatch\*\*\* \*\*\*repair\*\*\* in Schizosaccharomyces pombe requires the mutL homologous gene pms1: molecular cloning and functional analysis.

AN 97403304 MEDLINE

DN 97403304

TI \*\*\*Mismatch\*\*\* \*\*\*repair\*\*\* in Schizosaccharomyces pombe requires the mutL homologous gene pms1: molecular cloning and functional analysis.

AU Schar P; Baur M; Schneider C; Kohli J

CS Institute of Medical Radiobiology, University of Zurich, Switzerland..  
schaer@imr.unizh.ch

SO GENETICS, (1997 Aug) 146 (4) 1275-86.

Journal code: FNH. ISSN: 0016-6731.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

OS GENBANK-X96581; GENBANK-U13696; GENBANK-U28724; GENBANK-M29688;  
GENBANK-U13695; GENBANK-U07343; GENBANK-U07187

EM 199801

EW 19980104

AB Homologues of the bacterial mutS and mutL genes involved in DNA \*\*\*mismatch\*\*\* \*\*\*repair\*\*\* have been found in organisms from bacteria to humans. Here, we describe the structure and function of a newly identified Schizosaccharomyces pombe that encodes a predicted amino acid sequence of 794 residues with a high degree of homology to MutL related proteins. On the basis of its closer relationship to the eukaryotic "PMS" genes than to the "MLH" genes, we have designated the S. pombe homologue pms1. Disruption of the pms1 gene causes a significant increase of spontaneous mutagenesis as documented by reversion rate measurements. Tetrad analyses of crosses homozygous for the pms1 mutation reveal a reduction of spore viability from > 92% to 80% associated with a

low proportion (approximately 50%) of meioses producing four viable spores and a significant, allele-dependent increase of the level of post-meiotic segregation of genetic marker allele pairs. The mutant phenotypes are consistent with a general function of pms1 in correction of mismatched base pairs arising as a consequence of DNA polymerase errors during DNA synthesis, or of \*\*\*hybrid\*\*\* DNA formation between homologous but not perfectly complementary DNA strands during \*\*\*meiotic\*\*\*  
\*\*\*recombination\*\*\*

L9 ANSWER 2 OF 10 CAPLUS COPYRIGHT 2000 ACS

TI Use of DNA repair-defective hosts for \*\*\*meiotic\*\*\*

\*\*\*recombination\*\*\* of partially homologous DNA sequences

AN 1997:679170 CAPLUS

DN 127:327455

TI Use of DNA repair-defective hosts for \*\*\*meiotic\*\*\*

\*\*\*recombination\*\*\* of partially homologous DNA sequences

IN Borts, Rhona Harriet; Louis, Edward John

PA Setratech S.a.r.l., Fr.; Borts, Rhona Harriet; Louis, Edward John

SO PCT Int. Appl., 34 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9737011	A1	19971009	WO 1997-GB875	19970327
W: JP, US				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
PRAI US 1996-14490		19960401		

AB A method of allowing the in vivo homologous recombination of partially homologous DNA sequences having up to 30 % of base mismatches using eukaryotic host cells in which DNA \*\*\*mismatch\*\*\* \*\*\*repair\*\*\* system is defective and maintaining the recombinants to the point where they can segregate meiotically is described. Preferably the \*\*\*mismatch\*\*\* \*\*\*repair\*\*\* systems of the host cells are defective by mutation in the mutL or mutS genes (or both). The preferred host is a unicellular eukaryote such as a yeasts. In particular, a \*\*\*hybrid\*\*\* of Saccharomyces cerevisiae and S. paradoxus is a suitable hosts because the two organisms have weak sequence homol. but overall structural similarity of the genomes. Construction and characterization of a suitable \*\*\*hybrid\*\*\* of these two species is described.

L23 ANSWER 2 OF 9 CAPLUS COPYRIGHT 2000 ACS

TI Direct in vivo gene transfer to urological organs

AN 1999:570516 CAPLUS

DN 132:59704

TI Direct in vivo gene transfer to urological organs

AU Yoo, James J.; Soker, Shay; Lin, Lee F.; Mehegan, Kathryn; Guthrie, Paul D.; Atala, Anthony

CS Laboratory for Tissue Engineering and Cellular Therapeutics, Department, Children's Hospital and Harvard Medical School, Boston, MA, USA

SO J. Urol. (Baltimore) (1999), 162(3, Pt. 2), 1115-1118

CODEN: JOURAA; ISSN: 0022-5347

PB Lippincott Williams & Wilkins

DT Journal

LA English

AB Patients with urol. disorders may benefit from gene based therapy. The authors investigated the feasibility of delivering exogenous genes into urol. tissues in vivo using direct in vivo electrotransfection. Gene transfer to rat kidneys, testes and bladders was accomplished via direct local injection of pGL3/luciferase and .beta.-galactosidase reporter gene constructs, followed by an elec. pulse ranging from 55 to 115 ms. at 100 V. Direct injection of DNA without an elec. pulse served as the control. The transfected and nontransfected organs were retrieved and analyzed by luciferase activity assay, histochem. and immunocytochem. staining for .beta.-galactosidase, and reverse transcription polymerase chain reaction with primers specific for .beta.-galactosidase mRNA. There was significant luciferase activity 1, 3 and 5 days after direct in vivo electrotransfection in kidneys and testes, and after 3, 5, 7 and 10 days in bladders. Pos. .beta.-galactosidase enzyme activity and .beta.-galactosidase immunoreactivity were obsd. in the transfected renal tubular cells, testicular interstitial and germ cells, and uroepithelial bladder layer. Reverse transcription-polymerase chain reaction products of the transfected organs were noted, indicating the successful transcription of mRNA. This study demonstrates that direct in vivo electrotransfection is a feasible method of transient gene delivery into intact urol. organs. Its apparent safety and relative simplicity suggest that direct in vivo electrotransfection may be useful clin.

L23 ANSWER 1 OF 9 CAPLUS COPYRIGHT 2000 ACS

TI Transfection and \*\*\*transfer\*\*\* of male vertebrate \*\*\*germ\*\*\*  
\*\*\*cells\*\*\* for generation of transgenic species and \*\*\*gene\*\*\*  
\*\*\*therapy\*\*\*

AN 1999:354383 CAPLUS

DN 131:1452

TI Transfection and \*\*\*transfer\*\*\* of male vertebrate \*\*\*germ\*\*\*  
\*\*\*cells\*\*\* for generation of transgenic species and \*\*\*gene\*\*\*  
\*\*\*therapy\*\*\*

IN Readhead, Carol W.; Winston, Robert; Hovatta, Outi

PA Cedars-Sinai Medical Center, USA; Imperial College of Science, Technology  
and Medicine

SO PCT Int. Appl., 38 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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PI	WO 9925863	A1	19990527	WO 1998-US24238	19981113
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI,					

CM, GA, GN, GW, ML, MR, NE, SN, TD, TG  
 AU 9914061 A1 19990607 AU 1999-14061 19981113  
 PRAI US 1997-65825 19971114  
 US 1997-PV65825 19971114  
 WO 1998-US24238 19981113  
 AB Claimed is a compn. for in vivo transfection of vertebrate male  
 \*\*\*germ\*\*\* \*\*\*cells\*\*\* comprises a nucleic acid or transgene, a  
 \*\*\*gene\*\*\* delivery system, and optionally a protective internalizing  
 agent such as an endosomal lytic agent, a virus or a viral component,  
 which is internalized by cells along with the transgene and which enhances  
 \*\*\*gene\*\*\* \*\*\*transfer\*\*\* through the cytoplasm to the nucleus of  
 the male \*\*\*germ\*\*\* \*\*\*cell\*\*\*. A pharmaceutical prepn. and a  
 transfer kit utilize the compn. A method for introducing a polynucleotide  
 into vertebrate male germ cells comprises the administration of the compn.  
 to a vertebrate. A method for isolating or selecting transfected cells  
 utilizes a reporter gene, and a method for administering transfected male  
 germ cells utilizes male germ cells which have been transfected in vitro.

L28 ANSWER 9 OF 11 BIOSIS COPYRIGHT 2000 BIOSIS

TI Gene transfer by manipulation of primordial germ cells in the chicken.  
 AN 1994:505493 BIOSIS  
 DN PREV199497518493  
 TI Gene transfer by manipulation of primordial germ cells in the chicken.  
 AU Han, Jae Y. (1); Shoffner, R. N.; Guise, K. S.  
 CS (1) Dep. Animal Sci. Technol., Coll. Agric. Life Sci., Seoul National  
 Univ., Suwon 441-744 South Korea  
 SO Asian-Australasian Journal of Animal Sciences, (1994) Vol. 7, No. 3, pp.  
 427-434.  
 ISSN: 1011-2367.

DT Article

LA English

AB The primordial \*\*\*germ\*\*\* \*\*\*cells\*\*\* (PGCs) were  
 \*\*\*transfected\*\*\* in vitro and expressed the exogenous RSVLTR/beta-G2  
 plasmid, suggesting that PGC is a possible vector for direct \*\*\*gene\*\*\*  
 \*\*\*transfer\*\*\* into the germ line. \*\*\*Transfection\*\*\* efficiency of  
 cell suspensions containing PGCs was 1.5% by liposome mediated DNA  
 \*\*\*transfection\*\*\*. By microinjection of the \*\*\*transfected\*\*\* PGCs  
 into the host germinal crescent, PGCs migrated via blood vessel to the  
 future gonad and these \*\*\*transfected\*\*\* PGCs resulted in the  
 RSVLTR/beta-G2 expression in the gonad. The results from the seeding of  
 PGCs on the chorioallantoic membrane were insufficient to test the  
 hypothesis that PGCs can penetrate or invade the chorioallantoic membrane  
 for transport via the circulatory system.

L28 ANSWER 4 OF 11 MEDLINE DUPLICATE 3

TI In vivo gene transfer to mouse spermatogenic cells by deoxyribonucleic  
 acid injection into seminiferous tubules and subsequent electroporation.  
 AN 1999047421 MEDLINE  
 DN 99047421  
 TI In vivo gene transfer to mouse spermatogenic cells by deoxyribonucleic  
 acid injection into seminiferous tubules and subsequent electroporation.  
 AU Yamazaki Y; Fujimoto H; Ando H; Ohyama T; Hirota Y; Noce T



CS a Mitsubishi Kasei Institute of Life Sciences, Machida, Tokyo 194-8511, Japan.

SO BIOLOGY OF REPRODUCTION, (1998 Dec) 59 (6) 1439-44.

Journal code: A3W. ISSN: 0006-3363.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 199903

EW 19990301

AB An in vivo \*\*\*gene\*\*\* \*\*\*transfer\*\*\* technique for living mouse testes was used to develop a novel transient expression assay system for transcriptional regulatory elements of spermatogenic specific \*\*\*genes\*\*\*. The combination of DNA injection into seminiferous tubules and subsequent in vivo electroporation resulted in an efficient and convenient assay system for \*\*\*gene\*\*\* expression during spermatogenesis. The \*\*\*transfer\*\*\* of the firefly luciferase reporting \*\*\*gene\*\*\* driven by the Protamine-1 (Prm-1) enhancer region revealed a significant increase in the activity of the reporter enzyme. Histochemical studies of the \*\*\*transfer\*\*\* of the lacZ \*\*\*gene\*\*\* driven by the Prm-1 enhancer showed specific lacZ expression only in haploid spermatid cells in adult testes, corresponding with the expression pattern of endogenous Prm-1. We were able to detect long-lasting transgene expression in the \*\*\*transfected\*\*\* spermatogenic cells. A group of spermatogenic differentiating cells maintained the \*\*\*transfected\*\*\* lacZ expression after more than 2 mo of \*\*\*transfection\*\*\*, suggesting that spermatogenic stem cells and/or spermatogonia could also incorporate foreign DNA and that the transgene could be transmitted to the progenitor cells derived from a \*\*\*transfected\*\*\* proliferating \*\*\*germ\*\*\* \*\*\*cell\*\*\*.

L28 ANSWER 1 OF 11 MEDLINE

DUPLICATE 1

TI In vivo gene transfer to mouse spermatogenic cells using green fluorescent protein as a marker.

AN 2000092946 MEDLINE

DN 20092946

TI In vivo gene transfer to mouse spermatogenic cells using green fluorescent protein as a marker.

AU Yamazaki Y; Yagi T; Ozaki T; Imoto K

CS Laboratory of Humoral Information, National Institute for Physiological Sciences, Okazaki 444-8585, Japan.. yukiko@nips.ac.jp

SO JOURNAL OF EXPERIMENTAL ZOOLOGY, (2000 Feb 1) 286 (2) 212-8.

Journal code: I47. ISSN: 0022-104X.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 200004

EW 20000401

AB Combination of the DNA injection into seminiferous tubules and the subsequent in vivo electroporation (EP) has become an efficient and convenient assay system for spermatogenic-specific \*\*\*gene\*\*\* expression during spermatogenesis of mice. In this study, we made methodological modifications to enhance the \*\*\*transfection\*\*\* efficiency, and evaluated the possibility of this technique to generate

transgenic offspring using green fluorescent protein (GFP) as a marker. After the *in vivo* \*\*\*gene\*\*\* \*\*\*transfer\*\*\*, GFP expression could be monitored easily and repeatedly on the surface of the testis of live mice under fluorescent microscopy. The serial sections of the \*\*\*transfected\*\*\* testis revealed that transient expression of GFP was extended even in the innermost region of the testis uniformly, but confined to spermatogenic cells and Sertoli cells within the seminiferous tubules. Furthermore, long-lasting GFP expression could be detected in the spermatogenic cells even 2 months after EP. Natural mating with normal adult females revealed that 65% of the \*\*\*transfected\*\*\* males maintained fertilizable ability and could generate their offspring normally. Germ-line transmission of the GFP vector to the offspring was checked under fluorescent microscopy, but no transgenic offspring has been detected up to now. These results suggest that the application of additional techniques, such as cell sorting for GFP-positive \*\*\*germ\*\*\* \*\*\*cells\*\*\* followed by nuclear \*\*\*transfer\*\*\* to the oocytes, would make this method as a novel strategy for generating transgenic animals. J. Exp. Zool. 286:212-218, 2000. Copyright 2000 Wiley-Liss, Inc.

L28 ANSWER 3 OF 11 MEDLINE DUPLICATE 2

TI Direct in vivo gene transfer to urological organs.

AN 1999385625 MEDLINE

DN 99385625

TI Direct in vivo gene transfer to urological organs.

AU Yoo J J; Soker S; Lin L F; Mehegan K; Guthrie P D; Atala A

CS Department of Urology, Children's Hospital and Harvard Medical School, Boston, Massachusetts 02115, USA.

SO JOURNAL OF UROLOGY, (1999 Sep) 162 (3 Pt 2) 1115-8.

Journal code: KC7. ISSN: 0022-5347.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Abridged Index Medicus Journals; Priority Journals; Cancer Journals

EM 199912

EW 19991201

**AB PURPOSE:** Patients with urological disorders may benefit from \*\*\*gene\*\*\* based therapy. We investigated the feasibility of delivering exogenous \*\*\*genes\*\*\* into urological tissues in vivo using direct in vivo electrotransfection. **MATERIALS AND METHODS:** \*\*\*Gene\*\*\* \*\*\*transfer\*\*\* to rat kidneys, testes and bladders was accomplished via direct local injection of pGL3/luciferase and beta-galactosidase reporter \*\*\*gene\*\*\* constructs, followed by an electrical pulse ranging from 55 to 115 msec at 100 V. Direct injection of deoxyribonucleic acid without an electrical pulse served as the control. The \*\*\*transfected\*\*\* and nontransfected organs were retrieved and analyzed by luciferase activity assay, histochemical and immunocytochemical staining for beta-galactosidase, and reverse transcription polymerase chain reaction with primers specific for beta-galactosidase messenger ribonucleic acid. **RESULTS:** There was significant luciferase activity 1, 3 and 5 days after direct in vivo electrotransfection in kidneys and testes, and after 3, 5, 7 and 10 days in bladders. Positive beta-galactosidase enzyme activity and beta-galactosidase immunoreactivity were observed in the \*\*\*transfected\*\*\* renal tubular cells, testicular interstitial and \*\*\*germ\*\*\* \*\*\*cells\*\*\*, and uroepithelial bladder layer. Reverse

transcription-polymerase chain reaction products of the  
\*\*\*transfected\*\*\* organs were noted, indicating the successful  
transcription of messenger ribonucleic acid. CONCLUSIONS: This study  
demonstrates that direct in vivo electrotransfection is a feasible method  
of transient \*\*\*gene\*\*\* delivery into intact urological organs. Its  
apparent safety and relative simplicity suggest that direct in vivo  
electrotransfection may be useful clinically.

L32 ANSWER 1 OF 3 SCISEARCH COPYRIGHT 2000 ISI (R)

TI Effect of the topoisomerase-II inhibitor etoposide on meiotic  
recombination in male mice

AN 2000:96696 SCISEARCH

GA The Genuine Article (R) Number: 278XP

TI Effect of the topoisomerase-II inhibitor etoposide on meiotic  
recombination in male mice

AU Russell L B (Reprint); Hunsicker P R; Hack A M; Ashley T

CS OAK RIDGE NATL LAB, DIV LIFE SCI, OAK RIDGE, TN 37831 (Reprint); YALE  
UNIV, SCH MED, DEPT GENET, NEW HAVEN, CT 06510

CYA USA

SO MUTATION RESEARCH-GENETIC TOXICOLOGY AND ENVIRONMENTAL MUTAGENESIS,  
(24

JAN 2000) Vol. 464, No. 2, pp. 201-212.

Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM,  
NETHERLANDS.

ISSN: 1383-5718.

DT Article; Journal

FS LIFE

LA English

REC Reference Count: 39

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Unlike other chemicals that have been tested in mammalian germ cells,  
the type-II topoisomerase inhibitor etoposide exhibits significant  
mutagenicity in primary spermatocytes. Because this is the cell stage  
during which meiotic recombination normally occurs, and because  
topoisomerases play a role in recombination, we studied the effect of  
etoposide on crossing-over in male mice. Exposure to those meiotic  
prophase stages (probably early to mid-pachytene) during which  
specific-locus deletion mutations can be induced resulted in decreased  
crossing-over in the p-Tyr(c) interval of mouse chromosome 7. Accompanying  
cytological studies with fluorescent antibodies indicated that while there  
was no detectable effect on the number of recombination nodules (MLH1  
foci), there were marked changes in the stage of appearance and  
localization of RAD51 and RPA proteins. These temporal and spatial protein  
patterns suggest the formation of multiple lesions in the DNA after MLH1  
has already disappeared from spermatocytes. Since etoposide blocks  
religation of the cut made by type II topoisomerases, repair of DNA damage  
may result in rejoining of the original DNA strands, undoing the  
reciprocal exchange that had already occurred and resulting in reduced  
crossing-over despite a normal frequency of MLH1 foci. Crossing-over could  
conceivably be affected differentially in different chromosomal regions.  
If, however, the predominant action of etoposide is to decrease homologous  
meiotic recombination, the chemical could be expected to increase  
nondisjunction, an event associated with human genetic risk; Three periods  
in spermatogenesis respond to etoposide in different ways. Exposure of (a)

late differentiating spermatogonia (and, possibly, preleptotene spermatocytes) results in cell death; (b) early- to mid-pachytene induces specific-locus deletions and crossover reduction; and, (c) late pachytene-through-diakinesis leads to genetically unbalanced conceptuses as a result of clastogenic damage. (C) 2000 Elsevier Science B.V. All rights reserved.

L36 ANSWER 18 OF 30 MEDLINE DUPLICATE 12  
TI Gene transfer and expression in progeny after intravenous DNA injection into pregnant mice.  
AN 95291313 MEDLINE  
DN 95291313  
TI Gene transfer and expression in progeny after intravenous DNA injection into pregnant mice.  
AU Tsukamoto M; Ochiya T; Yoshida S; Sugimura T; Terada M  
CS Genetics Division, National Cancer Center Research Institute, Tokyo, Japan.  
SO NATURE GENETICS, (1995 Mar) 9 (3) 243-8.  
Journal code: BRO. ISSN: 1061-4036.  
CY United States  
DT Journal; Article; (JOURNAL ARTICLE)  
LA English  
FS Priority Journals  
EM 199509  
AB Several methods that enable foreign genes to be transferred directly into \*\*\*germ\*\*\* \*\*\*cells\*\*\* and adult animals have been developed, which have stimulated great interest in manipulating genes in \*\*\*vivo\*\*\*. However, there have been no methods available for introducing genes into fetuses. We report here that a single intravenous injection of expression plasmid: lipopolyamine complexes into pregnant mice resulted in successful gene \*\*\*transfer\*\*\* into the embryos. The transgenes thus introduced were expressed in the fetuses and newborn progeny. This simple and new method of gene \*\*\*transfer\*\*\* into embryos will facilitate rapid analysis of transgene effects in the fetuses and will be useful for studying gene-deficient animal models to gain transgene functions at desired stages of embryogenesis.

L36 ANSWER 10 OF 30 MEDLINE DUPLICATE 8  
TI Adenovirus-mediated gene transfer to rat testis in vivo.  
AN 97167625 MEDLINE  
DN 97167625  
TI Adenovirus-mediated gene transfer to rat testis in vivo.  
AU Blanchard K T; Boekelheide K  
CS Department of Pathology and Laboratory Medicine, Brown University, Providence, Rhode Island 02912, USA.  
SO BIOLOGY OF REPRODUCTION, (1997 Feb) 56 (2) 495-500.  
Journal code: A3W. ISSN: 0006-3363.  
CY United States  
DT Journal; Article; (JOURNAL ARTICLE)  
LA English  
FS Priority Journals  
EM 199706  
EW 19970604

AB To study transgene expression in the adult rat testis in \*\*\*vivo\*\*\* , an adenovirus vector carrying a lacZ transgene with a nuclear localization signal was used as a marker. The adenovirus vector was first tested on rat Sertoli cell- \*\*\*germ\*\*\* \*\*\*cell\*\*\* cocultures in vitro; it efficiently mediated transgene expression in Sertoli cells but not \*\*\*germ\*\*\* \*\*\*cells\*\*\* . This vector was then delivered to the interstitial compartment of adult rat testes by intratesticular injection, resulting in Leydig cells expressing the transgene. Alternatively, delivering the vector to the intratubular compartment by rete testis injection resulted in expression of the transgene by Sertoli cells of the seminiferous epithelium and principal cells of the epididymis. In \*\*\*vivo\*\*\* , each cell type expressed the transgene by 2 days postinfection, and expression persisted for at least 10 days; however, later time points were associated with a loss of transgene expression and focal interstitial inflammation. This study documents the ability of adenovirus to mediate gene \*\*\*transfer\*\*\* to specific testicular cells, providing a powerful tool to study the short-term effects of specific genes on spermatogenesis in \*\*\*vivo\*\*\* .

L36 ANSWER 9 OF 30 MEDLINE DUPLICATE 7  
 TI In vivo gene transfer to mouse spermatogenic cells by deoxyribonucleic acid injection into seminiferous tubules and subsequent electroporation.  
 AN 1999047421 MEDLINE  
 DN 99047421  
 TI In vivo gene transfer to mouse spermatogenic cells by deoxyribonucleic acid injection into seminiferous tubules and subsequent electroporation.  
 AU Yamazaki Y; Fujimoto H; Ando H; Ohyama T; Hirota Y; Noce T  
 CS a Mitsubishi Kasei Institute of Life Sciences, Machida, Tokyo 194-8511, Japan.  
 SO BIOLOGY OF REPRODUCTION, (1998 Dec) 59 (6) 1439-44.  
 Journal code: A3W. ISSN: 0006-3363.  
 CY United States  
 DT Journal; Article; (JOURNAL ARTICLE)  
 LA English  
 FS Priority Journals  
 EM 199903  
 EW 19990301  
 AB An in \*\*\*vivo\*\*\* gene \*\*\*transfer\*\*\* technique for living mouse testes was used to develop a novel transient expression assay system for transcriptional regulatory elements of spermatogenic specific genes. The combination of DNA injection into seminiferous tubules and subsequent in \*\*\*vivo\*\*\* electroporation resulted in an efficient and convenient assay system for gene expression during spermatogenesis. The \*\*\*transfer\*\*\* of the firefly luciferase reporting gene driven by the Protamine-1 (Prm-1) enhancer region revealed a significant increase in the activity of the reporter enzyme. Histochemical studies of the \*\*\*transfer\*\*\* of the lacZ gene driven by the Prm-1 enhancer showed specific lacZ expression only in haploid spermatid cells in adult testes, corresponding with the expression pattern of endogenous Prm-1. We were able to detect long-lasting transgene expression in the transfected spermatogenic cells. A group of spermatogenic differentiating cells maintained the transfected lacZ expression after more than 2 mo of transfection, suggesting that spermatogenic stem cells and/or spermatogonia could also incorporate foreign DNA and that the transgene could be transmitted to the progenitor

cells derived from a transfected proliferating \*\*\*germ\*\*\* \*\*\*cell\*\*\*

L36 ANSWER 6 OF 30 MEDLINE DUPLICATE 5  
TI Establishment of pluripotent cell lines from vertebrate species--present status and future prospects.  
AN 2000075319 MEDLINE  
DN 20075319  
TI Establishment of pluripotent cell lines from vertebrate species--present status and future prospects.  
AU Prelle K; Vassiliev I M; Vassilieva S G; Wolf E; Wobus A M  
CS Department of Molecular Animal Breeding and Genetics, Gene Centre, Ludwig Maximilian University, Munich, Germany.. k.prelle@gen.vetmed.uni-muenchen.de  
SO Cells Tissues Organs, (1999) 165 (3-4) 220-36. Ref: 152  
Journal code: DCO. ISSN: 1422-6405.  
CY Switzerland  
DT Journal; Article; (JOURNAL ARTICLE)  
General Review; (REVIEW)  
(REVIEW LITERATURE)  
LA English  
FS Priority Journals  
EM 200004  
EW 20000402  
AB Pluripotent embryonic stem (ES) cells are undifferentiated cell lines derived from early embryos and are capable of unlimited undifferentiated proliferation in vitro. They retain the ability to differentiate into all cell types including \*\*\*germ\*\*\* \*\*\*cells\*\*\* in chimeric animals in \*\*\*vivo\*\*\*, and can be induced to form derivatives of all three germ layers in vitro. Mouse ES cells represent one of the most important tools in genetic research. Major applications include the targeted mutation of specific genes by homologous recombination and the discovery of new genes by gene trap strategies. These applications would be of high interest for other model organisms and also for livestock species. However, in spite of tremendous research activities, no proven ES cells colonizing the germ line have been established for vertebrate species other than mouse and chicken thus far. This review summarizes the current status of deriving pluripotent embryonic stem cell lines from vertebrates and recent developments in nuclear \*\*\*transfer\*\*\* technology, which may provide an alternative tool for genetic modification of livestock animals.  
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